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Supplemental Material

Sex-Specific Effects of Prenatal and Early Life Inorganic and Methylated Arsenic Exposure on Atherosclerotic Plaque Development and Composition in Adult ApoE$^{-/-}$ Mice

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Table S1: Effect of arsenicals on plasma cholesterol, triglycerides, HDL, LDL, ALT and AST levels of apoE$^{-/-}$ male mice.

Groups	Cholesterol mmol/L	Triglycerides mmol/L	HDL mmol/L	LDL mmol/L	ALT U/L	AST U/L
Control (5)	11.4 ± 2.2	1.2 ± 0.2	1.7 ± 0.2	9.1 ±2.0	56 ± 19	129 ± 32
NaAsO$_2$ (7)	14.1 ± 3.8	1.4 ± 0.5	1.8 ± 0.3	11.6 ± 3.7	53 ± 10	104 ± 24
MMA V (4)	15.5 ± 1.9	1.6 ± 0.5	2.0 ± 0.2	12.8 ± 1.7	56 ± 15	117 ± 23
MMA III (5)	17.3 ± 3.3	1.9 ± 0.6	2.2 ± 0.3	14.3 ± 3.0	65 ± 20	111 ± 46
DMA V (4)	15.2 ± 2.5	1.3 ± 0.3	2.0 ± 0.3	12.5 ± 2.2	61 ± 13	144 ± 46

Note: The numbers in parentheses represent the number of animals used for all the parameters in each of the exposure groups.

Table S2: Effect of arsenicals on plasma cholesterol, triglycerides, HDL, LDL, ALT and AST levels of apoE$^{-/-}$ female mice.

Groups	Cholesterol mmol/L	Triglycerides mmol/L	HDL mmol/L	LDL mmol/L	ALT U/L	AST U/L
Control (4)	13.3 ± 0.4	1.5 ± 0.3	1.7 ± 0.1	10.8 ± 0.3	44 ± 5	104 ± 15
NaAsO$_2$ (5)	13.3 ± 4.1	1.4 ± 0.5	1.6 ± 0.2	10.9 ± 3.8	42 ± 7	111 ± 32
MMA V (4)	18.2 ± 1.4	1.6 ± 0.5	1.7 ± 0.2	15.7 ± 1.7	38 ± 7	124 ± 44
MMA III (4)	14.6 ± 6.2	1.6 ±0.6	1.7 ± 0.3	11.1 ± 4.5	43 ± 16	114 ± 20
DMA V (4)	17.0 ±2.8	1.1 ± 0.2	1.6 ± 0.1	14.8 ± 3.0	58 ± 23	149 ± 89

Note: The numbers in parentheses represent the number of animals used for all the parameters in each of the exposure groups.

Table S3: Comparison between the control groups of apoe$^{-/-}$ and apo$^{-/-}$ As3mt$^{-/-}$ DKO mice

Genotype		Arch				Sinus		
		(% of total arch area)		(% of total sinus area)		mm^2		
		Relative change (95% CI)	p-value	Relative change (95% CI)	p-value	Relative change (95% CI)	p-value
DKO	All offspring	4.4 (0.7, 8.2)	0.02	5.0 (0.7, 9.3)	0.02	0.04 (0, 0.09)	0.06
	Females	7.2 (2.9, 11.5)	<0.01	7.1 (-0.6, 14.8)	0.07	0.05 (-0.02, 0.13)	0.17
	Males	3.9 (-0.4, 8.2)	0.07	2.7 (-0.1, 5.6)	0.06	0.04 (0, 0.07)	0.06

Table S4: Mean and standard deviations for the apoE^{-/-} dataset pertaining to the lesion areas in aortic arch and the aortic sinus.

Groups	Lesion area [a] (% total arch area) Mean $\pm$ SD			Lesion area [b] (%total sinus area) Mean $\pm$ SD			Lesion area [c] (mm^2) Mean $\pm$ SD		
	All litters	Females	Males	All litters	Females	Males	All litters	Females	Males
Control	2.7$\pm$1.8	3.0$\pm$2.1	2.6$\pm$1.5	9.0$\pm$5.5	12.8$\pm$5.2	5.6$\pm$2.9	0.06$\pm$0.04	0.09$\pm$0.04	0.04$\pm$0.02
NaAsO$_2$	5.4$\pm$3.0	5.6$\pm$3.0	5.3$\pm$3.0	13.0$\pm$7.1	14.5$\pm$7.9	10.5$\pm$4.4	0.08$\pm$0.06	0.09$\pm$0.06	0.07$\pm$0.05
MMA III	13.3$\pm$6.3	15.1$\pm$7.2	10.2$\pm$2.1	19.8$\pm$10.8	22.4$\pm$11.3	14.5$\pm$6.9	0.15$\pm$0.14	0.19$\pm$0.15	0.06$\pm$0.03
MMA V	10.8$\pm$4.5	12.0$\pm$3.9	9.9$\pm$5.0	12.7$\pm$6.6	14.1$\pm$5.1	11.5$\pm$7.6	0.07$\pm$0.04	0.07$\pm$0.02	0.07$\pm$0.05
DMA V	10.9$\pm$4.5	10.7$\pm$4.5	11.1$\pm$4.6	15.6$\pm$7.1	19.2$\pm$8.6	13.3$\pm$5.5	0.12$\pm$0.08	0.15$\pm$0.12	0.09$\pm$0.05

Note: The averages and standard deviations were calculated for complete litters as well as separately for female and male offspring for plaque size in the aortic arch and the aortic sinus pertaining to all the groups. The graphical data distribution is depicted in figures 1 and 2 and the data adjusted by sex and litter is presented in Tables 2 and 3.
SD, standard deviation
[a] Percentage of oil red O (ORO) stained area over the total arch area; Number of apoE^{-/-} litters (N)= Control: 12, NaAsO$_2$: 9, MMA III: 4, MMA V: 5; DMA V: 5
[b] Percentage of ORO-stained lesion area over the total sinus area; Number of apoE^{-/-} litters (N)= Control: 12, NaAsO$_2$: 9, MMA III: 4, MMA V: 4; DMA V: 4
[c] Absolute lesion area as measured by the ORO-staining; Number of apoE^{-/-} litters (N)= Control: 12, NaAsO$_2$: 9, MMA III: 4, MMA V: 4; DMA V: 4

Table S5: Mean and standard deviations for the apoE$^{-/-}$ dataset pertaining to the macrophage, lipid, smooth muscle cell and collagen content in the aortic sinus.

Groups	Macrophages [a] (% of sinus lesion area) Mean ± SD			Lipids [b] (% of sinus lesion area) Mean ± SD			Smooth Muscle Cells [c] (% of sinus lesion area) Mean ± SD			Collagen [d] (% of sinus lesion area) Mean ± SD		
	All litters	Females	Males	All litters	Females	Males	All litters	Females	Males	All litters	Females	Males
Control	27.8±5.4	29.3±3.7	26.0±7.0	32.4±6.3	34.2±7.2	30.3±3.9	30.7±6.4	26.7±3.2	33.7±7.4	39.5±7.4	35.2±3.1	44.3±8.1
NaAsO$_2$	24.3±4.3	24.6±3.0	24.1±4.7	35.6±3.4	36.1±3.3	35.0±3.4	25.8±4.0	27.5±3.8	23.7±3.0	39.2±6.8	42.7±6.0	35.0±5.3
MMA III	25.2±3.7	24.7±4.0	26.0±2.8	34.5±4.9	36.0±5.0	33.1±4.8	27.3±4.0	25.7±4.0	29.3±3.4	40.6±6.8	41.7±5.3	39.5±8.3
MMA V	26.9±4.2	27.4±5.1	24.1±3.6	35.5±4.6	43.7±3.6	43.7±5.2	27.4±4.3	27.0±3.9	27.9±5.5	38.5±6.0	37.9±6.3	39.0±5.9
DMA V	23.9±5.7	23.5±6.4	26.3±5.6	41.7±4.4	34.2±4.1	37.1±4.1	24.7±3.5	24.7±4.5	24.8±2.4	34.1±8.4	41.1±6.7	28.15±3.5

Note: The averages and standard deviations were calculated for macrophage, lipid, smooth muscle cell and collagen content in the control and treated groups. These values are presented for complete litters as well as for female and male offspring. The graphical data distribution is depicted in figures 3 and 4 and the data adjusted by sex and litter is presented in Tables 4 and 5.

SD, standard deviation

[a] Percentage of moma-2 antibody stained area over the total lesion area in the aortic sinus as measured by the intensity of fluorescent signal at 488 nm; Number of apoE$^{-/-}$ litters (N)= Control: 6, NaAsO$_2$: 3, MMA III: 4, MMA V: 3; DMA V: 4

[b] Percentage of oil red O (ORO)-stained area over the total lesion area in the aortic sinus; Number of apoE$^{-/-}$ litters (N)= Control: 6, NaAsO$_2$: 3, MMA III: 4, MMA V: 3; DMA V: 4

[c] Percentage of total SMAα antibody-stained area over the total lesion area in the aortic sinus as measured by the intensity of fluorescent signal at 488 nm; Number of apoE$^{-/-}$ litters (N)= Control: 6, NaAsO$_2$: 3, MMA III: 4, MMA V: 3; DMA V: 3

[d] Percentage of picrosirius red-stained area over the total lesion area in the aortic sinus; Number of apoE$^{-/-}$ litters (N)= Control: 6, NaAsO$_2$: 3, MMA III: 3, MMA V: 3; DMA V: 3

Table S6: Mean and standard deviations for the apoE$^{-/-}$ As3mt$^{-/-}$ dataset pertaining to the lesion areas in aortic arch and the aortic sinus.

Groups	Lesion area [a] (% total arch area) Mean $\pm$ SD			Lesion area [b] (%total sinus area) Mean $\pm$ SD			Lesion area [c] (mm^2) Mean $\pm$ SD		
	All litters	Females	Males	All litters	Females	Males	All litters	Females	Males
Control	7.1$\pm$6.9	7.7$\pm$6.4	6.6$\pm$7.6	14.0$\pm$7.6	20.1$\pm$6.0	8.2$\pm$4.2	0.10$\pm$0.08	0.13$\pm$0.09	0.07$\pm$0.07
NaAsO$_2$	9.6$\pm$5.4	10.8$\pm$5.1	8.2$\pm$5.4	13.0$\pm$5.4	14.8$\pm$6.3	10.9$\pm$3.2	0.09$\pm$0.1	0.11$\pm$0.13	0.07$\pm$0.07

Note: The averages and standard deviations were calculated for complete litters as well as separately for female and male offspring for plaque size in the aortic arch and the aortic sinus pertaining to all the groups. The graphical data distribution is depicted in figures 5 and 6 and the data adjusted by sex and litter is presented in Tables 6 and 7.
SD, standard deviation; Number of DKO litters (N)= Control: 4, NaAsO$_2$: 4

[a] Percentage of oil red O (ORO) stained area over the total arch area
[b] Percentage of ORO-stained lesion area over the total sinus area
[c] Absolute lesion area as measured by the ORO-staining